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STIMULATION OF THE ANTERIOR PITUITARY BY CERTAIN CINCHONINIC ACID DERIVATIVES[1]

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Received for publication Dec. 7, 1949

Previous investigations in this laboratory have shown certain derivatives of cinchoninic acid to be effective antidiuretics when administered to experimental animals with induced water diuresis or to patients afflicted with diabetes insipidus (1). These substances, as well as other derivatives of cinchoninic acid which are devoid of antidiuretic activity, are also capable of inhibiting the secretion of phenol red by the kidney (2).[3] Some derivatives of cinchoninic acid have long been known to cause an increased rate of excretion of uric acid when administered to man (3, 4, 5). As the excretion of uric acid is known to be directly influenced by the secretions of the adrenal cortex and indirectly by the secretions of the anterior pituitary (6, 7), it appeared possible that these actions of derivatives of cinchoninic acid might be in part effected through the pituitary-adrenal system. Stimulation of the adrenals by secretions or extracts of the anterior pituitary is attended by a decrease in their ascorbic acid content (8) and evidence has been adduced that this decrease may be taken as an indication of secretory activity (9). Thus, injection of adrenocorticotropic hormone, epinephrine, or histamine into intact rats causes a marked decrease in the ascorbic acid content of their adrenals. Since the effect of the latter two drugs is abolished by hypophysectomy, it appears that these substances stimulate the pituitary to exert its specific regulatory control over the ad-

[1] This investigation has been aided by grants from the U. S. Public Health Service and The Life Insurance Medical Research Fund.

[2] Eli Lilly Fellow in Pharmacology and Experimental Therapeutics.

[3] Further studies of the antidiuretic and phenol red inhibiting effects of many more cinchoninic acid derivatives than are given in the two publications quoted have been made. A description of the preparation of these compounds and the results of tests of their biological activities will be published.

renals (10, 11). Should derivatives of cinchoninic acid act in similar fashion upon the pituitary, injections of these substances into rats should cause an analogous decrease in the ascorbic acid content of their adrenals and this action should be abolished by hypophysectomy. Accordingly, in the hope of furthering our understanding of the pharmacological actions of derivatives of cinchoninic acid, the effect of certain of these substances and some related antipyretic drugs upon the ascorbic acid content of the adrenals of both intact and hypophysectomized rats has been examined.

Male rats of the Sprague-Dawley or Wistar strains were used. The rats weighed from 140 to 348 grams. In any one experiment the weight range was much narrower. The drugs were injected subcutaneously as the sodium salts. Control rats were injected with physiological saline solution. Two hours after injection, the animals were deeply anesthetized with an intraperitoneal injection of sodium pentobarbital (1 cc. of a ten per cent solution). Three minutes after injection both adrenals were removed, cleaned of fat and weighed. Rats, hypophysectomized by the parapharyngeal approach, were used forty-eight hours after operation. The heads of all these animals were fixed in formalin. Dissection with the aid of a lens revealed that the anterior lobe was absent in all animals, and that there was no gross injury to the base of the brain.

Ascorbic acid was determined by the method of Roe and Kuether (12).

The results of the experiments are summarized in table 1. The data disclose that, when administered in sufficiently large doses, a number of the cinchoninic derivatives examined cause a marked decrement in the ascorbic acid content of the adrenals of intact rats. Of the several substances of this type examined, two of the more potent, 3-hydroxy-2-phenylcinchoninic acid and 3-hydroxy-2-phenylquinoline-4,8-dicarboxylic acid proved to be ineffective in hypophysectiomized rats. From these observations it is evident that neither of these substances acts directly upon the adrenals. Their probable mode of action, and presumably that of the other derivatives of cinchoninic acid having the same action, would seem to be stimulation of the anterior pituitary and consequent indirect stimulation of the adrenal cortex.

The manner in which drugs of the type under discussion effect

TABLE 1

The Concentration of Ascorbic Acid in the Adrenal Glands of Male Rats Two Hours after Subcutaneous Injection of Derivatives of Cinchoninic Acid

NAME OF COMPOUND	DOSE	NO. OF RATS	CONCENTRATION OF ASCORBIC ACID
Normal Sprague-Dawley rats			
	mgm./kgm.		*mgm./100 gm. adrenal*
Controls		8	404 ± 7.1*
3-hydroxy-2-phenylcinchoninic acid	200	6	212 ±12.4
3-hydroxy-2-phenylcinchoninic acid	100	7	340 ±36.6
3-hydroxy-2-phenylcinchoninic acid	50	8	355 ±28.3
3-hydroxy-2-phenylquinoline-4,8 dicarboxylic acid	50	7	274 ± 9.0
3-hydroxy-2-phenylquinoline-4,8 dicarboxylic acid	25	8	246 ± 7.7
3-hydroxy-2-phenylquinoline-4,8 dicarboxylic acid	12	8	332 ±44.9
Hypophysectomized Sprague-Dawley rats			
Controls		8	347 ±14.4
3-hydroxy-2-phenylcinchoninic acid	200	8	331 ±25.9
3-hydroxy-2-phenylquinoline-4,8 dicarboxylic acid	100	7	348 ±11.5
Normal Wistar rats			
Controls		19	352 ± 9.0
3-hydroxy-2-phenylcinchoninic acid	200	9	234 ±23.9
3-hydroxy-2-phenylcinchoninic acid	100	13	289 ±17.0
3-hydroxy-2-phenylcinchoninic acid	50	5	337 ±33.8
2-hydroxy-3-phenylcinchoninic acid	400	4	362 ±30.3
2-phenylcinchoninic acid (cinchophen)	400	3	203 ±11.6
2-phenylcinchoninic acid (cinchophen)	200	3	214 ±13.6
2-phenylcinchoninic acid (cinchophen)	100	4	302 ±33.1
2-phenylcinchoninic acid (cinchophen)	50	4	380 ± 5.6
2-hydroxycinchoninic acid	400	4	271 ±44.7
2-methylcinchoninic acid	400	4	343 ±20.3
2-hydroxy-3-methylcinchoninic acid	400	4	338 ±26.4
7-chloro-3-hydroxy-2-methylcinchoninic acid	100	5	240 ±12.6
7-chloro-3-hydroxy-2-methylcinchoninic acid	50	4	376 ±31.5
7-chloro-3-hydroxy-2-methylcinchoninic acid	25	4	357 ±35.2
3-hydroxy-2-methylquinoline-4,8-dicarboxylic acid	100	4	248 ±29.9
3-hydroxy-2-methylquinoline-4,8-dicarboxylic acid	50	4	298 ±20.0
3-hydroxy-2-methylquinoline-4,8-dicarboxylic acid	25	5	312 ±20.2
3-hydroxy-2-methylquinoline-4,8-dicarboxylic acid	12	5	391 ±27.8
6,8-dichloro-3-hydroxy-2-phenylcinchoninic acid	100	4	329 ±49.3
2-hydroxybenzoic acid (salicylic acid)	300	4	187 ±23.0
2-hydroxybenzoic acid (salicyclic acid)	100	4	319 ±16.2

TABLE 1—*Concluded*

NAME OF COMPOUND	DOSE	NO. OF RATS	CONCENTRATION OF ASCORBIC ACID
Normal Wistar rats—*Continued*			
	mgm./kgm.		*mgm./100 gm. adrenal*
2-hydroxybenzoic acid (salicylic acid)	50	4	376 ±17.4
4-hydroxybenzoic acid	400	5	338 ±20.7
4-dimethylamino-1,5-dimethyl-2-phenyl-3-pyrazolone (aminopyrine)	400	3	231 ±15.3
4-dimethylamino-1,5-dimethyl-2-phenyl-3-pyrazolone (aminopyrine)	200	3	226 ± 4.7
4-dimethylamino-1,5-dimethyl-2-phenyl-3-pyrazolone (aminopyrine)	100	4	287 ±11.6
4-dimethylamino-1,5-dimethyl-2-phenyl-3-pyrazolone (aminopyrine)	50	4	304 ±21.3

* Each figure given in this column is the mean with its standard error.

stimulation of the pituitary cannot be deduced from the data presently at hand. Whatever the precise locus of action may be, it is of interest to examine the relation of the ability of drugs to effect a decrease in the ascorbic acid content of rat adrenals to other aspects of their pharmacological behavior.

As shown in table 1, under the conditions of our experiments, both sodium salicylate and aminopyrine cause a decrease in the ascorbic acid content of the adrenals. This may be a general property of the coal tar antipyretics, for by means of techniques somewhat different from those employed by us, Svirbley (13) and Frommel, Piquet, Cuenod and Loutfi (14) working with rats and guinea pigs respectively have found that the administration of aminopyrine, acetanilid and chemically related antipyretics cause a decrease in the ascorbic acid content of the adrenals. This is of considerable interest in view of the known central nervous system actions of antipyretic drugs. In this connection, one may note that p-hydroxybenzoic acid, an isomer of salicylic acid which is essentially devoid of antipyretic properties, is without effect upon the ascorbic acid content of rats adrenals.

It has long been known that ingestion of salicylates by man enhances the rate of excretion of uric acid. Analogous observations do not appear to have been made with aminopyrine although the chemi-

cally related antipyretic drug, antipyrine, is said to cause an increased uric acid excretion when administered to rabbits (15) and to dogs (16). In contrast to salicylic acid, the isomeric p-hydroxybenzoic acid is said to be without influence upon the daily excretion of uric acid by man (17, 18) although this has been questioned by Quick (19) who found the latter substance to possess uricosuric activity when collections of urine were made for only a few hours following ingestion of the drug. Quick accounts for this discrepancy by noting that unlike salicylic acid, p-hydroxybenzoic acid in man is rapidly converted to the readily excreted p-hydroxyhippuric acid. A similar "detoxification" may account for the inactivity of p-hydroxybenzoic acid observed in the present experiments.

Information concerning the uricosuric activity of all of the derivatives of cinchoninic acid employed in the present investigation is lacking. Of the five derivatives found to cause a decrease in the ascorbic acid content of rats adrenals in the present experiments, two, namely 2-phenylcinchoninic acid (3) and 3-hydroxy-2-phenylcinchoninic acid (5) are known to exhibit marked uricosuric properties when administered to man. Such properties are not exhibited by 2-methylcinchoninic acid (5) which, in the maximum doses used, failed to cause a decrease in the ascorbic acid content of rat adrenals in our experiments. These facts coupled with the known effects of adrenocorticotropin on the excretion of uric acid (6, 7) and our observation that 3-hydroxy-2-phenylcinchoninic acid and 3-hydroxy-2-phenylquinoline-4,8-dicarboxylic acid are without effect upon the adrenals of hypophysectomized rats, strongly suggest that certain pharmacological properties of these and related drugs, including their uricosuric activity, are largely mediated by their action upon the anterior pituitary.

SUMMARY

A number of cinchoninic acid derivatives, salicylic acid and aminopyrine have been found to decrease the adrenal ascorbic acid of rats. The anterior pituitary gland has been shown to be an essential mediator of this effect.

We wish to thank Sara Ann Verplanck and Irene T. Payne for technical assistance.

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FURTHER OBSERVATIONS ON THE ANTIDIURETIC EFFECT OF CINCHONINIC ACID DERIVATIVES[1]

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In a previous communication (1) it was reported that when administered to dogs 3-hydroxy-2-methylcinchoninic acid and 3-hydroxy-2-phenylcinchoninic acid inhibit water diuresis. Such antidiuretic activity is not exhibited by the isomeric acids, 2-hydroxy-3-methylcinchoninic acid and 2-hydroxy-3-phenylcinchoninic acid, nor by the structurally related acids, 2-methylcinchoninic acid and 2-phenylcinchoninic acid. Accordingly, it appears that in this series of compounds, antidiuretic activity is determined by the basic structure exemplified in 3-hydroxycinchoninic acid. Difficulties encountered in the preparation of this hitherto undescribed substance have precluded an examination of its pharmacological behavior. If, however, this deduction is valid, then one may reasonably expect that the introduction of substituents into the structure of 3-hydroxy-2-methylcinchoninic acid or its congener, 3-hydroxy-2-phenylcinchoninic acid should modify the antidiuretic potency of these compounds in a fashion more or less predictable on the basis of purely chemical considerations. Since the hydroxy group in position-3 of the cinchoninic acid molecule appears to be an integral part of the basic structure which determines antidiuretic activity, ablation of the phenolic properties of this group by conversion to the methyl ether should result in a loss of antidiuretic activity. In agreement with this, as will be shown later, 3-methoxy-2-phenylcinchoninic acid does not display such activity. Similarly, replacement of the phenolic hydroxy group by an amino group results in a complete loss of antidiuretic activity. This is evidenced by the inactivity of both 3-aminocinchoninic acid and 3-amino-2-phenylcinchoninic acid.

[1] This investigation has been aided by a grant from the U. S. Public Health Service, and by a grant from the Life Insurance Medical Research Fund. Certain chemicals have been purchased on a grant from the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry of the American Medical Association.

It is well established that the introduction of substituents into the proper position of phenols may markedly alter their phenolic properties. Accordingly, one would anticipate that the introduction of substituent groups into the benzenoid ring of either 3-hydroxy-2-methylcinchoninic acid or 3-hydroxy-2-phenylcinchoninic acid should alter the antidiuretic potency of the parent compounds in a fashion consonant with the expected effect of such substituents upon the properties of the phenolic group in position-3 of these compounds. Because of this and because of the well known electron-attracting effect of the chlorine atom in organic molecules we have prepared and examined the relative antidiuretic activity of a series of chlorinated derivatives of the two 3-hydroxycinchoninic acids previously shown (1) to possess such activity. In the case of both of these acids one should expect on theoretical grounds that the most marked effect upon the phenolic properties of the hydroxy group would follow the introduction of a chlorine atom into position-7 of their molecules. In conformity with this expectation the 7-chloro derivatives of both 3-hydroxy-2-methylcinchoninic acid and 3-hydroxy-2-phenylcinchoninic acid were found to be more potent antidiuretics than either of the parent compounds or any of their isomeric derivatives.

Because, on theoretical grounds, a carboxy group in position-8 of the cinchoninic acid molecule might be expected to exert an influence upon the phenolic group in position-3 somewhat comparable to that exercised by a chlorine atom in position-7, 3-hydroxy-2-methylquinoline,4,8-dicarboxylic acid and 3-hydroxy-2-phenylquinoline-4,8-dicarboxylic acid were prepared and examined. These substances were found to be the most potent antidiuretics of the series of acids so far examined. Because of the pronounced activity of these two substances, the antidiuretic activity of the structurally related quinoline-8-carboxylic acid was also investigated. This substance failed to exhibit antidiuretic activity.

PREPARATION OF COMPOUNDS

3-Nitrocinchoninic acid was obtained in 8 per cent of the theoretical yield by the condensation of isatin with nitromethane in the manner described by Colonna (2). This substance was reduced in conventional fashion with ammonium sulfide to yield 3-aminocinchoninic acid iden-

tical with that obtained by the use of iron powder and acetic acid as described by Colonna. 3-Amino-2-phenylcinchoninic acid was prepared in essentially the manner outlined by Berlingozzi and Marzella (3). 3-Methoxy-2-phenylcinchoninic acid was obtained by the interaction of 3-hydroxy-2-phenylcinchoninic acid (1) and methyliodide, rather than by previously published (4, 5) methods of preparation involving the use of less readily available α-methoxyacetophenone. Quinoline-8-carboxylic acid was obtained by oxidation of 8-methylquinoline as described by Axe (6).

The heretofore undescribed chlorinated derivatives of 3-hydroxycinchoninic acid whose properties are summarized in Table 1 were all prepared by Pfitzinger (7) condensation of either acetylmethyl acetate or phenacyl acetate with the appropriately substituted isatin required to yield the desired product. The substituted isatins required for this purpose were in part prepared by Sandmeyer's (8) procedure and in part furnished by Dr. R. C. Elderfield of Columbia University. Two techniques initially utilized for the preparation of respectively 7-chloro-3-hydroxy-2-methylcinchoninic acid and 7-chloro-3-hydroxy-2-phenylcinchoninic acid which, in our hands, were found to afford excellent yields of these substances, have been utilized for the preparation of all of the other monochloro-derivatives of the two parent acids. For this reason, we shall describe in detail only the preparation of these two members of the two series of isomeric acids. When applied to the synthesis of the 3-hydroxycinchoninic acids derived from 6,8-dichloroisatin and from isatin-7-carboxylic acid, these techniques yielded inadequate quantities of the desired end products. Accordingly, in these instances it was found necessary to devise alternative preparative techniques which are described in Table 1.

Because of their relatively low solubility in common organic solvents, the majority of the cinchoninic acid derivatives prepared were purified by solution in alkali, subsequent precipitation by acid and then by washing until free of the acid anion. In some instances, this proved to be a tedious operation. The majority of the acids prepared appear to separate from aqueous solution as unstable hydrates which lose water more or less readily at room temperature. Accordingly, in each instance the precipitated product was allowed to dry at room temperature until easily friable and then dried to constant weight in an oven

at 105 to 110°. This procedure was adopted following the observation that when either freshly precipitated 3-hydroxy-2-phenylcinchoninic acid or its 7-chloro-derivative was dried in an oven without preliminary air drying, evidence of partial decomposition was detectable. The "melting points" recorded in Table 1 are really decomposition points. As such, their magnitude varies markedly with the rate of heating. In each instance where the melting point lies above 200°C., it was determined in an electrically heated metal block similar to the Linström (9) type of apparatus.

TABLE 1

Monochloro derivatives of 3-hydroxycinchoninic acids

SUBSTITUENTS	% N CALC.	% N FOUND	MELTING POINT	% YIELD
7-chloro-2-methyl $C_{11}H_8O_2NCl$	5.89	5.83	275–278°	72
5-chloro-2-phenyl $C_{16}H_{10}O_2NCl$	4.67	4.62	195–196°	55
6-chloro-2-phenyl $C_{16}H_{10}O_2NCl$	4.67	4.69	190–191°	63
7-chloro-2-phenyl $C_{16}H_{10}O_2NCl$	4.67	4.70	213–215*	82
8-chloro-2-phenyl $C_{16}H_{10}O_2NCl$	4.67	4.63	168–169†	66

* When inserted in bath preheated to 206°.

† Recrystallized from a mixture of ethanol and heptane.

3-Methoxy-2-phenylcinchoninic Acid. A mixture of 5.3 gm. of 3-hydroxy-2-phenylcinchoninic acid, 5.2 gm. of anhydrous potassium carbonate, 75 cc. of acetone, previously dried over potassium carbonate, and 5 cc. of methyl iodide was gently refluxed together for 8 hours. After the mixture had refluxed for 2½ hours a further 5 cc. portion of methyl iodide was added through the condenser. The reaction mixture was filtered. The solid residue was extracted with 10 cc. of warm acetone and the combined acetone solutions were evaporated to dryness on a steam bath. The residue remaining was extracted with 35 cc.

of anhydrous ether. The ether extract was filtered and the ether removed by evaporation. The oily residue, presumably the methyl ester of the desired end product, was mixed with 10 cc. of 6 N sodium hydroxide solution and 30 cc. of alcohol and evaporated to dryness on a steam bath. The residue remaining was dissolved in 50 cc. of water and the resulting solution was acidified to Congo red by the addition of 6 N hydrochloric acid. The precipitated solid was separated, washed with water and dried. Yield 4.4 g (78%). Upon recrystallization from boiling alcohol (15 cc. per gm.) a very pale yellow product, m. pt. 215.5–216.5° decomp., was obtained. As this melting point was considerably lower than that recorded in the literature (4, 5) and could not be raised by repeated crystallization from alcohol, the identity of the compound as 3-methoxy-2-phenylcinchoninic acid was established by its nitrogen content, calc. for $C_{17}H_{13}O_3N$, N = 5.02%, found N = 4.8%, and by cleavage to 3-hydroxy-2-phenylcinchoninic acid in the manner described by Dilthey and Thelen (4).

7-Chloro-3-hydroxy-2-methylcinchoninic Acid. To a suspension of 27.2 gm. (0.15 mol) of 6-chloroisatin in 100 cc. of water contained in a 500 cc. flask there was added 30 cc. of 7.2 M sodium hydroxide solution, and then 20.8 gm. (20 per cent excess) of technical acetylmethyl acetate were added dropwise with shaking. Following the addition of 95 cc. more of 7.2 M sodium hydroxide, the mixture was gently refluxed for 3 hours and then held over night (18 hrs.) at room temperature. The reaction mixture was diluted with 500 cc. of water, filtered through a bed of Celite and with continuous stirring acidified to Congo red by the dropwise addition of 6 N hydrochloric acid. After standing over night the precipitated product was collected on a Buchner funnel, washed with three 50 cc. portions of water and dissolved in a solution of 14 gms. of sodium bicarbonate in 400 cc. of water. The resulting solution was stirred for 10–15 minutes with 5 gm. of decolorizing carbon and filtered. The filtrate was heated to incipient boiling, removed from the flame, rendered acid to Congo red by the dropwise addition of 6 N hydrochloric acid, and allowed to cool spontaneously to room temperature. The product was twice washed by decantation, transferred to a Buchner funnel, washed with water until free of chloride ion, and then dried in the manner specified above.

7-Chloro-3-hydroxy-2-phenylcinchoninic Acid. To a solution prepared

by dissolving 27.2 gm. (0.15 mole) of 6-chloroisatin (10) in 190 cc. of warm 6 N sodium hydroxide contained in a 1 liter flask, there was added in order with shaking, a solution of 29.4 gm. (0.165 mole) of phenacyl acetate in 200 cc. of warm ethanol and a solution of 26.6 gm. of sodium hydroxide in 95 cc. of water. The mixture was held at room temperature over night and the ethanol was removed by distillation. The mixture was then heated under reflux on a steam bath for 4 hours, diluted with 285 cc. of water, cooled, stirred with decolorizing carbon for 15 minutes and filtered. The filtrate was made acid to Congo red by the addition of hydrochloric acid. The precipitated product was twice washed by decantation with water, transferred to a Buchner funnel, washed with 4 10 cc. portions of water and dissolved in a solution of 13 gm. of sodium bicarbonate in 500 cc. of water. This solution was filtered to remove a small amount of insoluble material and the product precipitated therefrom by the addition of 40 cc. of 6 N acetic acid. After a few hours at room temperature, the product was collected on a Buchner funnel, washed first with 3 10 cc. portions of water, then with 2 10 cc. portions of ethanol, dried over night in vacuo at 37° and finally to constant weight at 105°.

6,8-Dichloro-3-hydroxy-2-methylcinchoninic Acid. Acetylmethyl acetate (14 gm.) was added dropwise with shaking to a solution prepared by dissolving 21.6 gm. (0.1 mole) of 5,7-dichloroisatin in a warm solution of 62 gm. of 86% potassium hydroxide in 200 cc. of water. The mixture was refluxed for 2 hours, allowed to remain at room temperature for 19 hours, diluted with 500 cc. of water and treated with hydrochloric acid until definitely acid to Congo red. After 3 hours the precipitate was collected and washed, first with water, then with acetone. The acetone washed product was dried in vacuo over sulfuric acid for 24 hours and suspended in 500 cc. of acetone. This suspension was heated to boiling, cooled and filtered. The crude product (20.7 gm., 76%) melted at 211–212°. A portion of this, upon recrystallization from aqueous ethanol (75 cc. of ethanol and 20 cc. of water per gm.), separated as almost colorless needles melting with decomposition at 250–251°. Analysis: Calc. for $C_{11}H_7O_3NCl_2$, N = 5.15%; found, N = 5.22%.

6,8-Dichloro-3-hydroxy-2-phenylcinchoninic Acid. A solution of 18.7 gm. (0.105 mole) of phenacyl acetate in 60 cc. of warm ethanol was

added to a solution of 21.6 gm. (0.1 mole) of 5,7-dichloroisatin prepared as specified in the preceding synthesis and the technique followed was exactly the same up to the time of precipitation with hydrochloric acid. The precipitated product was washed with water until free of chloride ions, dried first at room temperature and then at 105°. The crude product (19.8 gm., 60%) melted with decomposition at 195–197°. A portion of the product was purified by dissolving in warm acetone (50 cc. per gm.) adding water until the solution became faintly turbid, heating to boiling and allowing to crystallize over night. In this manner the product was obtained as bright yellow needles, m. pt. 203–204°, with decomposition. Analysis: Calc. for $C_{16}H_9O_3NCl_2$, N = 4.19%. Found, N = 4.20%.

3-Hydroxy-2-methylquinoline-4,8-dicarboxylic Acid. Acetylmethyl acetate (17.4 gm., 20% excess) was added to a solution of 28.7 gm. (0.15 mole) of 2,3-diketoindoline-7-carboxylic acid (11) in 150 cc. of 6 N sodium hydroxide solution contained in a 500 cc. flask. This mixture was held at room temperature for 2 hours, gently refluxed for 10 hours, cooled, diluted with 300 cc. of water and treated with 150 cc. of 6 N hydrochloric acid. After 2 hours at room temperature, the precipitated material was separated, washed with water and dissolved in a mixture of 150 cc. of cold water and 20 cc. of concentrated ammonia water. The resulting solution was stirred with 5 gm. of decolorizing carbon (Darco) for an hour, filtered, heated to boiling, precipitated by the dropwise addition of 47 cc. of hydrochloric acid and left over night at room temperature. The gelatinous precipitate was collected on a Buchner funnel, washed with water until free of chloride ion and dried, first on a porous tile, then in a vacuum desiccator for 4 days. The dry product was pulverized and suspended in 100 cc. of ethanol. The suspension was heated to boiling, cooled and filtered. The product was washed with 2 10 cc. portions of ethanol, dried over sulfuric acid in an evacuated desiccator for 2 days and then to constant weight at 70°. Yield 14.3 gm. (38.6%) of ivory colored powder which upon heating in a capillary tube darkened over the temperature range of 270–300° but did not melt at 310°. Analysis: Calc. for $C_{12}H_9O_5N$, N = 5.67%. Found N = 5.62%.

3-Hydroxy-2-phenylquinoline-4,8-dicarboxylic Acid. A solution of 32 gm. (0.18 mole) of phenacyl acetate in 100 cc. of warm ethanol were

added to a solution of 28.7 gm. (0.15 mole) of 2,3-diketoindoline-7-carboxylic acid in 150 cc. of 6 N sodium hydroxide contained in a 500 cc. flask. The mixture was gently refluxed for 10 hours, left at room temperature over night, and diluted with 300 cc. of water. Following removal of the ethanol by distillation, the mixture was diluted with water to a volume of approximately 1 liter and filtered through a bed of Filter-Cel to remove tarry by-products. The filtrate was treated with 150 cc. of 6 N hydrochloric acid and left at room temperature for 3 hours. The precipitated product was collected, washed with water, and dissolved in a mixture of 500 cc. of warm water and 30 cc. of 6 N ammonia water. The resulting solution was stirred with decolorizing carbon (3 gm. Darco), filtered, precipitated by the dropwise addition of 30 cc. of 6 N. hydrochloric acid and left at room temperature over night. The precipitated product was collected, washed with 5 10 cc. portions of water and dried at 37° for 3 days. The dry product was pulverized, boiled for a few minutes with 50 cc. of ethanol, separated from the solvent by filtration, washed with ethanol and dried, first in an evacuated desiccator over sulfuric acid for 24 hours and then at 105° for 2 hours. Yield 26.9 gm. (85.8%) of pale yellow product. Upon heating, the color began to darken at 239–240° becoming progressively darker as the temperature was raised until at 261–262° decomposition began with formation of a tarry mass which melted at 306–310°. Analysis: Calc. for $C_{17}H_{11}O_4N$, N = 4.53%. Found N = 4.56%.

METHODS

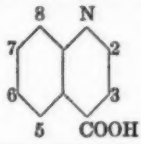
The antidiuretic effect of the compounds was tested on a water diuresis in the dog by the procedure previously used (1). The doses of the drugs were generally varied in multiples of two, and the minimum dose of drug which produced an unequivocal antidiuretic effect was determined. The minimal effective dose did not vary more than two-fold in different dogs, and it remained the same in any one dog at different times though the extent and duration of the response to any given dose varied from time to time in any one dog.

The acute toxicity of these compounds for white mice of the Carworth Farms CF-1 strain was determined by subcutaneous administration of a solution of the sodium salt. The mice were observed for

seven days after injection of the drug; however, most of the deaths occurred in the first twenty-four hours.

TABLE 2

The antidiuretic effect of cinchoninic acids



NO.	SUBSTITUENTS	ANTIDIURETIC ACTIVITY*
8	3-hydroxy-2-phenyl	1
11	8-carboxy-3-hydroxy-2-phenyl	160
12	8-carboxy-3-hydroxy-2-methyl	80
13	7-chloro-3-hydroxy-2-phenyl	5 or 10
14	7-chloro-3-hydroxy-2-methyl	5
16	6,8-dichloro-3-hydroxy-2-phenyl	2
15	5-chloro-3-hydroxy-2-phenyl	1
24	6-chloro-3-hydroxy-2-phenyl	1
17	6,8-dichloro-3-hydroxy-2-methyl	½
1	3-hydroxy-2-methyl	⅓
18	8-chloro-3-hydroxy-2-phenyl	¼ or ⅕
20	3-amino	—†
21	3-nitro	—
19	3-amino-2-phenyl	—
22	3-methoxy-2-phenyl	—‡
25	quinoline-8-carboxylic acid	—

* Activity is expressed in terms of 3-hydroxy-2-phenylcinchoninic acid.

† Inactive in dosage of 20 mgm. per kgm.

‡ Inactive in dosage of 5 mgm. per kgm.; higher doses caused marked toxic symptoms.

RESULTS AND DISCUSSION

Thirteen additional cinchoninic acid derivatives were tested for their antidiuretic action. The results are given in Table 2. Each active compound has been compared with a reference standard, 3-hydroxy-2-phenylcinchoninic acid, by determining the ratio of the minimal effective antidiuretic dose of the standard to the minimal effective antidiuretic dose of the compound under comparison. At least two dogs were used for testing each compound. All the dogs received on several

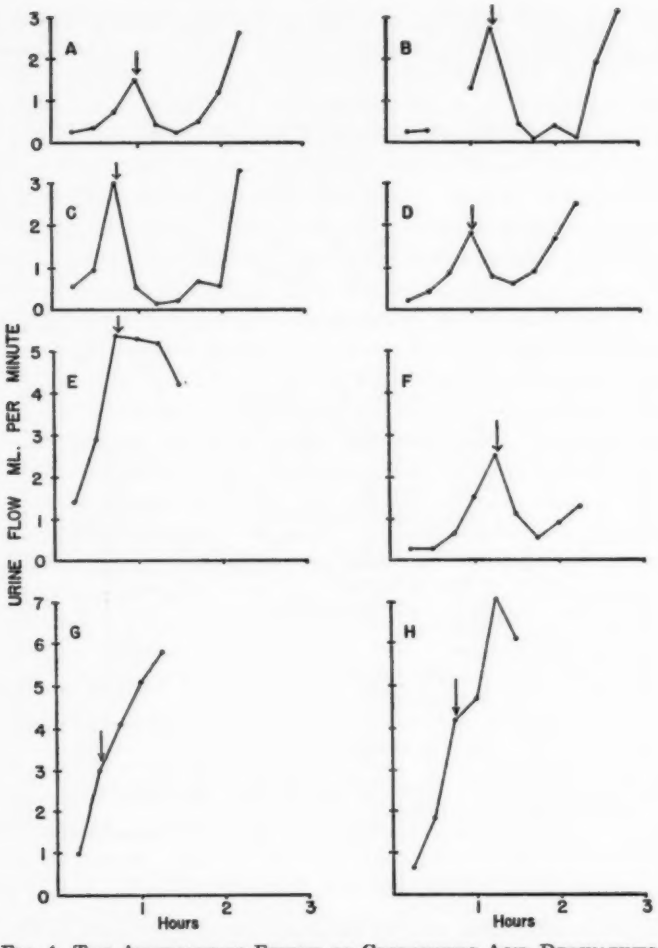


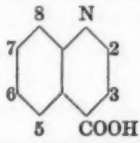
FIG. 1. THE ANTIDIURETIC EFFECT OF CINCHONINIC ACID DERIVATIVES IN THE DOG

C, E and G are respectively 5, 2.5 and 2.5 mgm. of 3-hydroxy-2-phenylcinchoninic acid per kgm. given intravenously as the sodium salt. A, B, D, F and H are respectively 1, 0.25, 0.06, 0.03 and 0.015 mgm. of 3-hydroxy-2-phenylquinoline-4,8 dicarboxylic acid per kgm. given intravenously as the disodium salt. The time of drug administration is indicated by the arrows. Diuresis was induced by 40 ml. water per kgm. given at zero time.

occasions different doses of 3-hydroxy-2-phenylcinchoninic acid, which has been used as the standard of comparison. With the standard, it has been found that a dose of 10 mgm. per kgm. in one dog always gave a response, but that half of this dose did not give a response. The magnitude and duration of the response may be independent of dosage.

TABLE 3

Acute subcutaneous toxicity of cinchoninic acids for mice



NO.	SUBSTITUENTS	DOSE (MGM. PER KGM.)				
		1600	800	400	200	100
		Dead/Total				
	None	0/10	0/5			
12	8-carboxy-3-hydroxy-2-methyl	0/18	0/16	0/8	0/8	0/8
2	2-hydroxy-3-methyl	0/12	0/10			
19	3-amino-2-phenyl	0/9	0/9			
9	2-hydroxy-3-phenyl	0/7	1/6			
22	3-methoxy-2-phenyl	5/8	0/16	0/8	0/8	
16	6,8-dichloro-3-hydroxy-2-phenyl	4/5	2/6	0/9		
17	6,8-dichloro-3-hydroxy-2-methyl		3/6	0/8		
10	2-phenyl		12/16	0/9		
18	8-chloro-3-hydroxy-2-phenyl		7/7	0/7	0/6	
23	3-ethoxy-2-phenyl	8/8	8/8	0/9		
11	8-carboxy-3-hydroxy-2-phenyl		13/18	1/8	0/8	0/8
1	3-hydroxy-2-methyl		8/8	6/8	0/8	0/8
14	7-chloro-3-hydroxy-2-methyl		4/4	12/13	0/8	0/9
8	3-hydroxy-2-phenyl		32/34	14/34	1/34	0/16
13	7-chloro-3-hydroxy-2-phenyl		16/16	10/16	5/16	0/8

The minimal effective dose of 3-hydroxy-2-phenylcinchoninic acid varied from 5 to 10 mgm. per kgm. in different dogs, but was quite constant in any one dog. We believe that the order of magnitude of the activity ratios is correct, but a two-fold difference may or may not be significant. The most active compound found thus far, 3-hydroxy-2-phenylquinoline-4,8-dicarboxylic acid is effective in a dosage of 0.03 mgm. per kgm.

In Figure 1 are given data showing how the activity of our most active compound was compared with our standard in one dog. Examination of this figure shows that 2.5 mgm. per kgm. of the standard gave no unequivocal response, but 5 mgm. did. It can also be seen that about the same response is obtained from 1, 0.25, 0.06, and 0.03 mgm. of the test drug, but that 0.015 mgm. is clearly inactive. Hence, the compound under test is 160 times as active as the standard.

The acute toxicity of sixteen cinchoninic acids has been tested by subcutaneous administration to white mice of the Carworth Farms CF-1 strain. The results are given in Table 3.

The introduction of a chlorine atom into position-7 leads to a marked increase in antidiuretic activity (Nos. 13 and 14, Table 2). This increased activity is not accompanied by an increase in toxicity for mice (Nos. 13, 14 and 8, Table 2). Likewise, the introduction of a carboxy group at position-8 leads to an increased antidiuretic activity and, in fact, gives the most potent antidiuretics of the series of acids so far examined (Nos. 11 and 12, Table 1). The toxicity of one of these most active compounds is no greater than that of the parent compound, while the other compound is the least toxic of any active cinchoninic acid derivative so far examined (Nos. 8, 11, and 12, Table 2). Substitution of chlorine in positions in the benzenoid ring other than position-7 has not resulted in any significant change in antidiuretic acitivity (Nos. 16, 15, 24, 17, and 18, Table 1). Thus, we see that the predictions presented in the introduction on the basis of purely chemical considerations have been surprisingly confirmed.

SUMMARY

1. Several new derivatives of cinchoninic acid have been synthesized and have been examined for their antidiuretic action.

2. The relative antidiuretic potency of these derivatives has been found to be more or less predictable on the basis of purely chemical considerations.

3. Increase in antidiuretic activity is not always accompanied by increased toxicity for mice.

We wish to thank Frances Fallon and Rose Johnson Randall for doing the nitrogen determinations.

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REDUCTION IN THE NUMBER OF CIRCULATING EOSINOPHILS FOLLOWING INDUCED ANOXEMIA[1]

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In view of the reports that stimulation of the adrenal cortex is of value in the treatment of various clinical conditions (1, 2, 3) and that anoxia is one form of stress causing adrenal cortical stimulation (4, 5), it was decided to study the effects of anoxia upon adrenal cortical activity in normal subjects, using a method of inducing anoxemia in which the exact degree and duration of anoxemia may be controlled (6). Reduction in the number of circulating blood eosinophils was used as the measure of adrenal activity, since it has been shown that both pituitary adrenocorticotropic hormone and 11-oxysteroids produce this effect (7).

METHOD

The studies were carried out on 11 healthy subjects, aged 19 to 30, who were physicians, medical students or technicians. They were allowed to have their meals and to participate in their usual activities both before and after the anoxemia test.

The desired degree and duration of anoxemia was obtained by the administration of a gas of variable low oxygen concentration with oximeter control, as described in detail elsewhere (6). The arterial oxygen saturation was lowered to between 80 and 70%; the total period of anoxemia ranged from 17 to 24 minutes. Toward the end of

[1] This investigation was supported (in part) by the United States Public Health Service and (in part) by funds made available for research in Preventive Medicine by Abbott Lab., Eli Lilly Co., Lederle Lab., Parke Davis and Co., Upjohn Co., the Ellis D. and Priscilla G. Slater Foundation, and (in part) by a grant from the American Cancer Society for studies on the relationship of pituitary hormones, thyroid hormone and the steroid hormones of the adrenal glands and gonads to normal and abnormal growth.

[2] Post-doctorate Fellow, United States Public Health Service.

five of the tests 5% CO_2 was added to the low oxygen gas mixture for purposes unrelated to the present study.

Twelve anoxemia tests were carried out in this manner. In a series of twelve control tests, eosinophil counts were taken in the usual way,

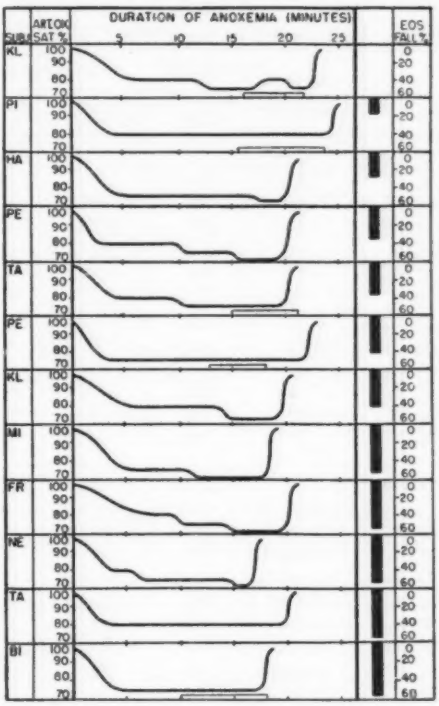

FIG. 1. DEGREE AND DURATION OF ANOXEMIA AND REDUCTION IN NUMBER OF EOSINOPHILS

┌——————┐ : Indicates period of 5% carbon dioxide administration

but the subjects were not exposed to anoxia. The number of eosinophils was determined in blood taken from the finger by direct count (8). The initial count was made in the morning approximately one-half hour before the anoxemia test. In both groups the second count was taken in the afternoon, approximately 4½ hours after the initial count.

OBSERVATIONS

The effect of anoxemia upon the circulating eosinophils is shown in figure 1 and table 1. It will be noted that the eosinophil count fell in

TABLE 1

Arterial Oxygen Saturation and Number of Eosinophils

GROUP	SUBJECT	ARTERIAL OXYGEN SATURATION*	EOSINOPHILS			
			Initial per cmm.	Final† per cmm.	Change per cmm.	Change
		per cent				*per cent*
Anoxemia	K. L.	75	124	125	+1	+1
	P. I.	80	99	83	−16	−16
	H. A.	72	605	430	−175	−29
	P. E.	70	345	225	−120	−35
	T. A.	75	155	100	−55	−36
	P. E.	75	485	283	−202	−42
	K. L.	72	133	77	−56	−42
	M. I.	70	168	79	−89	−53
	F. R.	70	66	31	−35	−53
	N. E.	70	312	139	−173	−55
	T. A.	80	130	55	−75	−57
	B. I.	75	188	81	−107	−57
	Mean		234	142	−92	−40
Control	T. A.	Normal	170	400	+230	+135
	P. E.	Normal	175	250	+75	+43
	D. E.	Normal	86	106	+20	+23
	T. A.	Normal	167	186	+19	+11
	P. E.	Normal	200	218	+18	+9
	B. R.	Normal	492	537	+45	+9
	B. I.	Normal	163	156	−7	−4
	P. I.	Normal	74	68	−6	−8
	M. I.	Normal	160	91	−69	−43
	F. R.	Normal	78	44	−34	−44
	K. L.	Normal	134	76	−58	−44
	F. R.	Normal	35	6	−29	−83
	Mean		158	178	+20	0

* Lowest arterial oxygen saturation reached during the test.

† Taken approximately 4½ hours after the initial count.

all but one of the instances where the subject was exposed to anoxemia, with a mean change of −40%. In contrast, the eosinophil count in

the control tests fluctuated in both directions, with a mean change of 0%. The *frequency* of tests showing an appreciable decrease in the number of eosinophils was also greater in the anoxemia group than in the control; in 10 of 12 instances anoxemia was followed by at least a 25% reduction in the eosinophils as contrasted to 4 of the 12 control tests.

DISCUSSION

These experiments indicate that the stress of anoxemia has a demonstrable effect upon the adrenal cortical activity of normal subjects, as measured by a reduction in the number of circulating eosinophils. The degree of anoxemia used was not extreme, and the period of exposure to a low oxygen gas was relatively brief, so it was not surprising that the reduction in eosinophils was less pronounced than that following such severe types of stress as intravenous epinephrine (9), electroconvulsive therapy (10), major surgery (11), normal labor or eclampsia (12). These studies raise the possibility that induced anoxemia, given frequently and perhaps for longer periods of time, is a practicable means of stimulating the adrenal cortex for purposes of therapy.

CONCLUSIONS

1. Exposure of normal subjects to a low oxygen gas producing arterial saturation of 70 to 80% led to a significant drop in eosinophil count.
2. This effect is thought to indicate an increase of adrenal cortical activity following anoxemia.

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A METHOD OF ADMINISTERING CARBON DIOXIDE AT A CONSTANT DEGREE OF INDUCED ANOXEMIA AND ITS CARDIOVASCULAR EFFECTS[1]

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INTRODUCTION

In previous communications, we have reported a method in which a *constant* degree of "anoxemia" (i.e. hypoxemia) may be induced by administering an inspired gas mixture of variable oxygen concentration (1, 2). No attempt was made, however, to compensate for the loss of carbon dioxide which is known to accompany induced anoxemia (3). It is the purpose of this present report, therefore, to describe a method whereby carbon dioxide can be administered with a gas of variable oxygen concentration and to demonstrate the value of such a procedure in differentiating the many similar effects (3–9) of induced hypoxemia from those of induced hypocapnia.

METHOD

The technique of inducing and maintaining a desired level of anoxemia has been described in detail elsewhere (2). In addition to the tanks of pure nitrogen and oxygen, two tanks—one containing 5% CO_2 in nitrogen and another containing 5% CO_2 in oxygen—were placed in circuit, as is shown diagramatically in Figure 1. The two tanks containing nitrogen were joined by a Y-shaped metal tubing to a single pressure-reducing valve; the two tanks containing the oxygen were arranged in a similar manner. With the tanks thus arranged, the inhaled gas could be made to contain 5% CO_2 by changing from the pure nitrogen and pure oxygen tanks to those containing the 5% CO_2,

[1] This investigation was supported by funds made available for research in the Dept. of Preventive Medicine by Abbott Lab., Eli Lilly Co., Lederle Lab., Parke Davis and Co., Upjohn Co., and the Ellis D. and Priscilla G. Slater Foundation.

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since any mixture of the two gases containing 5% CO_2 would necessarily contain the same concentration of CO_2. To verify this, a sample of gas, taken from the breathing bag while gas was flowing from the two tanks containing 5% CO_2, was analyzed by the Scholander method (10) and the carbon dioxide concentration was found to be 5%.

The arterial oxygen saturation was usually lowered in a step-like (2) manner down to levels of 70%. In most tests the pure oxygen and pure nitrogen gases were used at the beginning of the anoxemic period and the arterial saturation was lowered to the desired level before

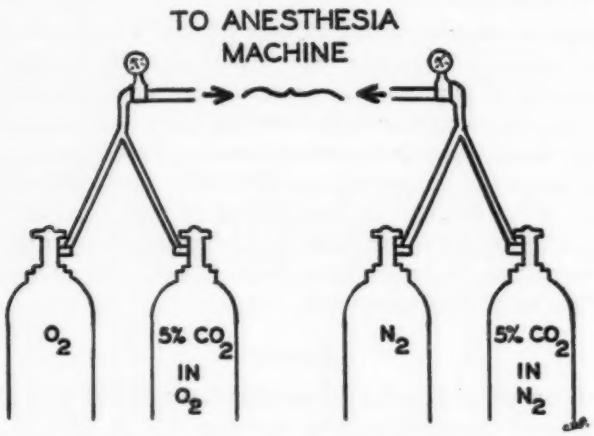

FIG. 1. ARRANGEMENT OF TANKS IN CIRCUIT

changing to the tanks containing the 5% CO_2. This was done so that the stability of the arterial saturation could be evaluated before the subject started to inhale the CO_2 gases. Several subjects were also given CO_2 at the beginning of the anoxemic period to determine if the arterial oxygen saturation could be lowered to, as well as maintained at, the desired level.

The subjects were all healthy white men ranging from 21 to 36 years of age. Blood pressure was measured by sphygmomanometer; heart rate was obtained from the electrocardiogram or from the radial pulse. Each subject rested for approximately forty-five minutes before inhaling the low oxygen gases.

OBSERVATIONS

26 anoxemia tests were done on 21 different subjects. Observations on the blood pressure, heart rate and electrocardiogram, at the various levels of arterial saturation, were made in 23 of the tests and are presented in Table 1.

(1) *The stability of the arterial saturation.* The arterial oxygen saturation *was just as stable with the 5% CO_2 gases* as with the simple oxygen-nitrogen mixtures. In most instances the arterial saturation did not fluctuate more than 1% during the period of inhalation of the CO_2 gases. This stability of the arterial saturation was independent of the degree or duration of anoxemia, the degree of hyperventilation due to the CO_2, or the time at which the CO_2 was administered (i.e. at the beginning or the end of the period of anoxemia).

An important factor in being able to keep the arterial saturation constant, at the desired level, was the anticipation of hyperventilation *before* the CO_2 gases were administered. Even if the subject's breathing was very shallow with the oxygen-nitrogen mixtures, the breathing bag was made *fully* distended with the O_2-N_2 gases before turning to the CO_2 gases. This prevented collapse of the bag, with probable instability of the arterial saturation, when the CO_2-induced hyperventilation started. Another point of importance in maintaining a constant arterial saturation with the CO_2 mixtures was the manipulation of the *oxygen* needle valve of the anesthesia machine to a greater extent than was necessary with the use of the oxygen-nitrogen mixtures (2). These increased changes of the oxygen flow rate were apparently necessary to alter the oxygen concentration of the gas in the *distended* breathing bag rapidly enough to maintain a constant degree of anoxemia.

It is of interest to note that with the tanks arranged in circuit, as shown in Figure 1, special valves to allow an instantaneous change from the pure oxygen-nitrogen tanks to the CO_2 tanks were not necessary. It had been expected that such valves would be needed to permit a continuous flow of gas to the subject while the 5% CO_2 was being introduced. The breathing bag and connecting tubing of the anesthesia machine, however, apparently contained enough of the remaining oxygen-nitrogen gases to allow the various tanks to be turned off, or on, in the usual manner.

TABLE 1

The individual cardiovascular response to anoxemia

SUBJ. NO.	ART. OX. SAT.	BLOOD PRESS.		HEART RATE (PER MIN.)	ELECTROCARDIOGRAM	
		Syst.	Diast.		RS-T dev. (I-IV incl.)	T (II)
	%	mm. *Hg.*	mm. *Hg.*		mm.	mm.
1	Normal	140	76	83		3
	80	148	76	103	½	2
	80*	150	78	94	0	2
	80	146	74	103	½	2
2	Normal	114	70	88		7
	75	122	76	97	1	5½
	75*	130	78	100	0	5
3	Normal	118	80	73		5
	75	118	78	75	½	4
	75*	116	80	75	½	3½
4	Normal	118	68	65		7
	75	124	74	88	0	4½
	70	130	74	91	0	4
	70*	134	73	86	1	3½
	75*	136	76	—	—	—
	75*	128	66	88	½	3½
5	Normal	128	76	88		4
	75	130	70	94	0	4
	75*	144	88	94	—	2½
6	Normal	130	66	—		—
	75	142	62	—	—	—
	75*	150	66	—	—	—
7	Normal	114	70	63		7
	70	120	78	90	2	2
	70*	138	70	91	1½	1
8	Normal	112	64	62		7
	80	126	64	81	1½	5
	75	128	67	88	2	3
	80*	120	68	85	2	3
	75*	134	66	97	3	3
9	Normal	116	62	63		5
	70	134	62	91	2½	2
	70*	136	60	100	½	½

TABLE 1—*Continued*

SUBJ. NO.	ART. OX. SAT.	BLOOD PRESS		HEART RATE (PER MIN.)	ELECTROCARDIOGRAM	
		Syst.	Diast.		RS-T dev. (I–IV incl.)	T (II)
	%	mm. *Hg.*	mm. *Hg.*		mm.	mm.
10	Normal	110	70	58		—
	75	118	76	77	—	—
	75*	110	68	65	—	—
11	Normal	116	72	63		6
	70	124	78	89	1½	3
	70*	136	81	90	1	3
12	Normal	112	74	70		4½
	75	120	80	81	0	4
	75*	122	80	74	0	4
13	Normal	128	70	75		3
	80	132	70	—	—	—
	70	134	66	106	2	1½
	70*	146	70	107	2½	1
	80*	146	76	—	—	—
14	Normal	128	78	—		—
	70	148	70	—	—	—
	70*	150	90	—	—	—
15	Normal	128	72	—		—
	80	128	80	—	—	—
	80*	128	76	—	—	—
16	Normal	100	60	78		3
	80	102	59	88	0	2½
	80*	102	70	86	0	2½
17	Normal	116	70	60		4½
	80	118	68	71	0	3
	75	114	68	73	0	2½
	80*	118	68	73	0	3
	75*	118	70	74	0	2½
18A	Normal	120	72	70		5
	75	116	68	79	½	4
	75*	122	68	87	½	3½
B	Normal	128	74	96		—
	85	126	74	100	—	—
	85*	124	72	98	—	—

TABLE 1—*Continued*

SUBJ. NO.	ART. OX. SAT.	BLOOD PRESS		HEART RATE (PER MIN.)	ELECTROCARDIOGRAM	
		Syst.	Diast.		RS-T dev. (I-IV incl.)	T (II)
	%	mm. *Hg.*	mm. *Hg.*		mm.	mm.
19A	Normal	110	68	68		4
	75	106	64	—	—	—
	75*	114	76	80	1	3½
B	Normal	112	64	72		—
	85*	113	74	72	—	—
	85	117	69	82	—	—
20A	Normal	120	72	72		—
	85	124	74	94	—	—
	80	128	76	100	—	—
	75	124	70	98	—	—
	80*	132	78	—	—	—
	75*	126	78	100	—	—
	85*	132	82	92	—	—
	92*	130	82	—	—	—
	96*	124	76	80	—	—
B	Normal	118	78	—		—
	90*	122	78	—	—	—
	87*	—	—	—	—	—
	85*	126	90	—	—	—
	80*	126	92	—	—	—
	75*	132	90	—	—	—
	80*	126	88	—	—	—
	85*	128	84	—	—	—

* Indicates that inspired gas contained 5% CO_2.

"RS-T deviation" refers to RS-T change from the control record obtained before the test.

(2) *Symptoms.* Four of the subjects (Nos. 12, 14, 15, and 19A) felt "faint" or "dizzy" while breathing the oxygen-nitrogen mixture. Changing to the 5% CO_2 gases resulted in prompt disappearance of symptoms in all four instances. Subject No. 15 was the most impressive of these. This subject, who was apprehensive, hyperventilated markedly before the test actually started even when breathing room air or pure oxygen. He was made anoxemic with the oxygen and nitrogen mixtures despite this hyperventilation. After five minutes of anoxemia,

his pulse suddenly became very weak and he appeared about to faint, even though his arterial oxygen saturation was still 80%. He was therefore given the CO_2 gases and within a minute regained his mental alertness and full pulse, allowing the anoxemia test to be continued for another 10 minutes and his saturation to be lowered to 75%. One of the subjects (No. 14) also had a sudden drop in the diastolic blood pressure which was reversed by the addition of CO_2.

TABLE 2

Cardiovascular changes with anoxemia

ART. OX. SAT.	BLOOD PRESS.		HEART RATE (PER MIN.)	ELECTROCARDIOGRAM	
	Syst.	Diast.		RS-T dev. (I–IV incl.)	T (II)
%	mm. Hg	mm. Hg		mm.	mm.
85	(3) +2.3	+2.3	(3) +12.0	(0) —	(0) —
85*	+3.0	+6.0	+7.3	—	—
80	(7) +5.3	+1.0	(4) +15.0	(4) 0.5	(4) −1.3
80*	+7.4	+4.3	+13.8	0.5	−1.8
75	(12) +4.5	+0.3	(10) +14.4	(7) 0.6	(8) −1.6
75*	+9.2	+3.2	+14.8	0.6	−2.1
70	(6) +11.7	+1.3	(5) +27.6	(5) 1.6	(5) −3.1
70*	+20.0	+4.0	+29.0	1.3	−3.8
85–70 incl.	(28) +6.0	+0.9	(22) +17.1	(16) 0.9	(17) −1.9
85–70 incl.*	+10.4	+4.0	+16.8	0.8	−2.5

* Indicates that inspired gas contained 5% CO_2.

Numbers in parentheses indicate number of comparisons made at the particular level of arterial oxygen saturation. Figures represent the average change for each group of observations. (+) or (−) indicates direction of change from control readings obtained on room air.

Of the other 17 subjects, none experienced any symptoms with inhalation of the 5% CO_2 mixtures nor objected to the hyperventilation brought on by its use.

(3) *Blood pressure and heart rate.* The blood pressure and heart rate changes noted at the various levels of anoxemia, with the oxygen-nitrogen or oxygen-nitrogen-carbon dioxide mixtures, are summarized in Table 2. Both the systolic and diastolic blood pressures were con-

sistently higher with carbon dioxide, especially at the level of 70% arterial saturation. There were some subjects, however, whose blood pressures were higher on the oxygen-nitrogen gases. In no instance did CO_2 inhalation cause so great an elevation of the blood pressure as to warrant termination of the test; the highest systolic reading obtained in any test with 5% CO_2 was 150 mm. Hg.

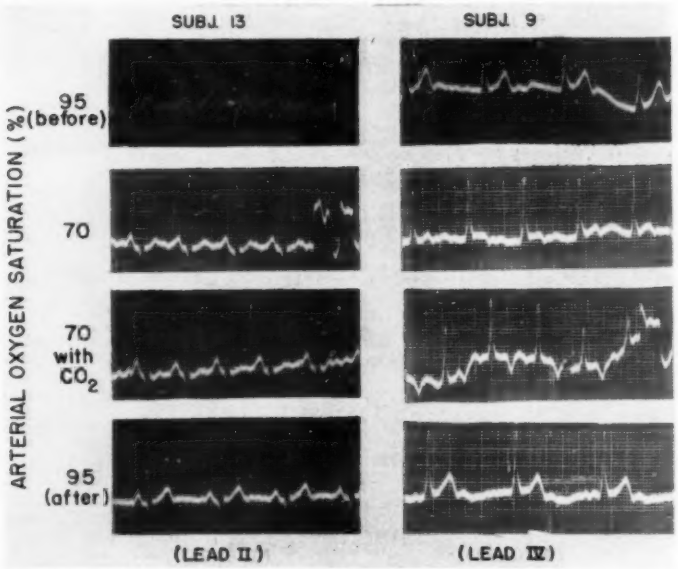

FIG. 2. T-WAVE CHANGES WITH ANOXEMIA

Electrocardiograms at "70 with CO_2" were taken approximately 5 minutes after those at "70".

In contrast to the blood pressure changes, the heart rate changes on CO_2 were not consistently different from those obtained with the oxygen-nitrogen mixtures. The average heart rate increase, for the levels of 85 to 70% arterial saturation, inclusive, was 17 beats per minute, either with or without the addition of 5% CO_2.

(4) *Electrocardiographic changes. RS-T deviation.* Sixteen comparative readings were made with CO_2 and the oxygen-nitrogen mixtures at the same levels of arterial saturation. The total RS-T deviation (leads I-CF4 incl.) did not differ significantly. The total deviation for

any one subject did not exceed 3 mm. with either gas. It is of interest to note that both with the oxygen-nitrogen or 5% CO_2 gases the RS-T deviation was appreciably greater at 70% arterial saturation than at 80 or 75% saturation.

T-Wave changes. The T-wave (lead II) with CO_2 was consistently *lower* than with the oxygen-nitrogen mixtures at the same degree of anoxemia. An example of this is illustrated in Figure 2. One subject (No. 9) showed actual *inversion* of the T-wave in lead IV with CO_2 but only *flattening* with the oxygen-nitrogen mixtures at the same saturation (Figure 2). It is of interest to note that the degree of T-wave lowering increased with the degree of anoxemia regardless of the type of gases used.

DISCUSSION

The ability to administer carbon dioxide while inducing anoxemia and still maintain a constant, stable level of the arterial oxygen saturation is in sharp contrast to the experiences with other methods. In an effort to differentiate the effects of hypocapnia from hypoxemia, various investigators administered carbon dioxide in the usual gases of *fixed* low oxygen concentration. Only occasionally could they obtain the same level of arterial oxygen saturation before and after the addition of carbon dioxide. When Barach and Steiner added 2 to 3% carbon dioxide (to 9 or 10% oxygen), the arterial oxygen saturation increased as much as 17% or decreased as much as 13% in the same subjects (3). Also, the variability of the arterial saturation among different subjects inhaling the same concentration of carbon dioxide in the same fixed low oxygen gas is marked, ranging between 47 and 73% in one group of 41 normal subjects (11).

In view of the physiological mechanisms involved in induced anoxemia, these changes and variability of the arterial saturation following the administration of CO_2 were to be expected for two reasons: (1) the degree of anoxemia varies in the same and different subjects breathing the same low oxygen gas (2), and (2) the addition of carbon dioxide to such a gas causes a further change of the saturation by changing the alveolar oxygen tension (12). It is apparent, therefore, that our method, which allows the oxygen concentration of the inhaled gas to be altered while carbon dioxide is being administered at a constant tension, overcomes these physiological difficulties.

The necessity for controlling the exact level of the arterial oxygen saturation in an anoxemia test has been discussed elsewhere where it was shown that a change of only 5% in the arterial saturation resulted in a significant change in the cardiovascular response (2, 13). In attempting to evaluate the effects of induced hypocapnia accompanying induced hypoxemia, therefore, it is essential to have exact control of the degree of hypoxemia when adding the carbon dioxide to the low oxygen gas.

Another advantage of the method of mixing the gases by this procedure is that a *fixed* concentration of carbon dioxide is delivered to the subject, regardless of the proportion of oxygen to nitrogen or the flow rates of these two gases. This is possible since each of the component gases of the mixture contains the same concentration of carbon dioxide. This procedure, therefore, allows concentrations of carbon dioxide other than 5% to be used with oximeter controlled anoxemia The oxygen-5% CO_2 and nitrogen-5% CO_2 tanks merely have to be replaced by tanks containing the required concentration of CO_2.

Five per cent was selected as the concentration of carbon dioxide in this series for two reasons: (1) 5% CO_2 causes marked hyperventilation, which was anticipated to be a major obstacle in maintaining a constant degree of anoxemia. Since the hyperventilation associated with 5% CO_2 did *not* prevent stable control of the arterial oxygen saturation, it may be inferred that the hyperventilation accompanying the use of lower concentrations of CO_2 also will not affect the stability of the arterial saturation. (2) Concentrations as high as 5% have been found to be a safe concentration to be administered with induced anoxemia (11). Also, relatively high concentrations of CO_2 in the inspired air tend to decrease the variability of the arterial blood *pH* (11) which, when combined with a controlled level of arterial blood oxygen saturation, must necessarily lead to a decreased variability of the *arterial oxygen tension* (14), control of which would appear to be the ultimate goal of a completely standardized induced anoxemia test.

Although the purpose of this report is primarily to describe a method, the observations made on our group of 21 normal subjects may be of some value in differentiating the effects of induced hypoxemia from those of induced hypocapnia. Fainting and collapse (4, 5, 6), lowering of the blood pressure (7, 8, 13), increase in heart rate (4, 9, 13), alteration of the RS-T segment (4, 9, 13) and T-wave lowering (9, 13) have

been attributed to either hypoxemia or hypocapnia or both. As stated previously, however, the various investigators were not able to maintain the same, constant degree of anoxemia when the carbon dioxide was added to the low oxygen gas. It was therefore impossible to state unequivocally that any physiological change following the addition of CO_2 was not due to an alteration in the arterial oxygen saturation.

The data obtained from our group of subjects, studied at the same levels of arterial saturation, both with and without the use of carbon dioxide (Tables 1 and 2), show that during induced anoxemia, hypocapnia, alone, may play a significant role in the production of some of the above changes. Every symptom encountered in our subjects could be attributed to hypocapnia. Addition of CO_2 to the gas mixture resulted in complete disappearance of such symptoms, allowing the test to be continued at the same or even a lower level of anoxemia. The blood pressure, both systolic and diastolic, was consistently higher with the addition of carbon dioxide, indicating that CO_2 loss also plays a part in lowering of the pressure. The addition of carbon dioxide had little effect, however, on the tachycardia or alteration of the RS-T segment produced by anoxemia. This suggests that anoxia itself is the direct cause of these changes. Contrary to the findings of other workers, carbon dioxide did not seem to prevent lowering of the T-wave. In fact its addition *increased* the amount of lowering.

It should be pointed out, however, that the concentration of carbon dioxide used, for reasons already mentioned, was set as 5%, and that this concentration probably *more than* compensates for the amount of CO_2 lost with induced anoxemia, thereby producing a state of mild acidosis. Inhalation of lower concentrations of carbon dioxide would possibly be adequate to replace the amount of carbon dioxide lost from the arterial blood during induced anoxemia.

SUMMARY

A method is described whereby carbon dioxide can be administered with a constant degree of induced anoxemia. It is a modification of a procedure previously described in which a low oxygen gas of variable oxygen concentration, with oximeter control, was used to induce and maintain a constant degree of anoxemia (1). The physiological importance of being able to administer carbon dioxide at a constant degree of anoxemia, in differentiating between the effects of hypoxemia

and hypocapnia, is discussed and data on the cardiovascular response of 20 normal subjects studied at various levels of anoxemia, both with and without carbon dioxide, are presented.

The author would like to express his appreciation to Doctors Caroline Bedell Thomas, Joseph L. Lilienthal, Jr., and Ross L. McLean for their helpful advice and assistance.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Bulletin)

Hematin Compounds and Bile Pigments. Their Constitution, Metabolism and Function. By R. LEMBERG and J. W. LEGGE. *Interscience Publishers, Inc., New York.* 1949, XXV + 749 pages. $15.00.

The Australian authors have reviewed a vast amount of information only sections of which had been available in monographs and reviews. The title and subtitle indicate the main subjects; the chlorophylls and related compounds are omitted. However, it would require copying almost the entire table of contents to show adequately the variety of topics discussed.

The principal values of the book are the following. (a) The authors' succinct summaries of what is known and what is debatable give good perspective of many topics some of which have been, or remain, in confusion because of inherent difficulties attending their investigation. (b) The book is virtually a guide to the literature. The bibliography contains 3182 references and, although the authors say that it is far from complete, this reviewer judges that it contains references to practically all the more important papers up to July, 1946. Notes added after the preparation of the type-script refer to several important papers to June, 1948. (c) In asmuch as Dr. Lemberg and his coworkers have illuminated the course of the breakdown of hemoglobin through intermediates, it is especially pleasing to have at first hand this review of that subject.

Readers of the BULLETIN will wish to know that the authors discuss briefly topics such as the porphyrias, sites of bile pigment formation, the anemias and many other subjects of particular interest to pathologist and clinician. A few of these sections of the book are the least satisfying for the following reason. At a given period some observations of biological events outrun the then existing, resolving abilities of the more exact sciences. Yet there are investigators who persist in applying whatever techniques of the exact sciences happen to be available. One cannot protest with good grace, especially because the wildcatters occasionally bring in gushers, but on the whole they leave a field in a mess. In dealing with a few of these cases Lemberg and Legge appear to have had their critical faculties smothered by masses of petty material. On the other hand, they are firm in dealing with several cases such as attempts to correlate varieties of jaundice with fine distinctions in the behavior of van den Bergh's reaction for bilirubin. While no one should go so far as to say there is nothing to this, scientific judgment rests firmly on the fact that the wildcatters outran systematic studies on the reaction they used and consequently left a mess of which Lemberg and Legge say: "There are few fields in biochemistry in which so much work has been done with so little success."

As a whole the book records a stately, systematic progress illuminated by the authors' keen appraisals.

The publishers have made a book of attractive appearance. The reviewer may complain that the book is too heavy (1.4 kilograms) to read with comfort when fascinated by the contents, one takes it to bed.

W. MANSFIELD CLARK

Clinical Interpretation of Laboratory Tests. By R. H. GOODALE. 622 pp. $6.50. *F. A. Davis, Philadelphia,* 1949.

This book was designed to aid in the selection and interpretation of the laboratory tests used in clinical medicine. In the first half of the volume, the various methods of laboratory diagnosis are discussed briefly but techniques are generally not described. The second part of the book deals with disease states and the procedures employed to study them. Because of the great number of tests and disorders included, the space devoted to each is small and the accounts are necessarily sketchy. In enumerating conditions in which a test gives abnormal results, often no mention is made of the fundamental defect responsible for the result. For example, several conditions are listed in which clot retraction is impaired, but it is not mentioned that thrombocytopenia is a feature of all of them. There are many unqualified statements which appear to be of questionable accuracy, and no attempt is made to evaluate critically the significance of abnormal results when they are found.

C. L. C.

Advances in Internal Medicine, volume 3. Edited by WILLIAM DOCK, M.D., and I. SNAPPER, M.D. 478 pp. $8.50. Published by *Interscience Publishers, New York,* 1949.

The selection of subjects and authors for this current summary of the changing picture of medicine continues to be excellent and broad in scope. The application of possibly the only completely rationally developed chemotherapy, that of B.A.L. to heavy metal poisoning by Drs. Longcope and Leutscher, is a natural first chapter. From this to current conception of hemolytic anemias, to factors governing the use of penicillin, and the clinical applications of streptomycin in tuberculosis, to name some of the outstanding subjects. Pinkerton describes and beautifully illustrates the histopathology of histoplasmosis. He deals adequately and carefully with the evidence for widespread infection of man with this fungus. In a concise chapter, Drs. Huggins and Talalay deal with "Diagnosis of Disease by Enzymatic Methods." Several of the authors point out in foot notes that the articles were written in the summer of 1947 and for that reason are not as up to date as desirable in a book published in 1949. For this the editors apologize.

F. B. B.

Child Psychiatry. By LEO KANNER. *Charles C. Thomas, Publisher, Springfield, Illinois,* 2nd Edition, 1948, pp. 752. $8.50.

This new and completely re-written second edition of a work that has deservedly

become a standard reference can best be described as a mature production. In it there is less anxiety that the whole field be covered, more security on the part of the author that his own clinical experience is not infrequently enough to rely on in determining what should be in the book and what should be left out, what should have much space and which topics might be given their just clinical due in a smaller number of lines of print. Concepts of diagnosis and treatment are more fully covered in this edition than in the last, while in general, there is rather less space devoted to particular behavior patterns. The greater reliance on literature in English as compared with the large number of German references of the first edition bespeaks the greater importance of the contributions of American and English psychiatry since 1935, the date of the first edition; perhaps also this change indicates a greater appreciation on the part of the author of the cultural factors that must be recognized if data collected in one milieu is to be taught to students learning in a very different one. The book is more mature also in the eclecticism the author shows, drawing as he does from the various schools of psychiatry and psychology— as well as from other disciplines—and welding them into a workable philosophy of diagnosis and treatment of childhood behavior disorders. There is less stress on the organic factor in disorder in this edition, probably because good books on the neurological diseases of children, notably Ford's which is frequently quoted, has made the inclusion of the material less necessary at this time of the new edition. The organic syndromes are still present in the book, however, and the material on epilepsy is very complete.

The format of the new book is an improvement on the old, though there are a few bothersome printing errors here and there. The print is larger and more readable, and the references are conveniently collected at the end of the subdivisions of each chapter.

This book will be indispensable to the child psychiatrist and to the pediatrician. Its practical orientation and its insistence on dealing with the child rather than his symptoms, with the person rather than with his "admission ticket" to the clinic, will make for sound work and protect from irresponsible theoretical fantasies. For many of us it will not replace our familiar friend, the first edition, but will be a companion piece to it.

P. V. L.

Histopathology of the Skin. By WALTER F. LEVER, M.D. 449 pp. $20.00. *J. B. Lippincott Company, Philadelphia.*

Dr. Lever's book comes at a very opportune time since no new editions have appeared on this subject for several years in this country. The book is printed on very good paper and there are 449 pages and 221 illustrations.

The author recognizes in his introduction the limitations of dermato-pathology and attempts to present it for what it is worth. This is a most worthy ambition, for, unfortunately, in many medical writings and older textbooks attempts to differentiate too sharply similar pathologic processes are made which the average student cannot see and which often do not exist.

The author has a clear brief chapter on embryology and normal histology of the skin, illustrated with photomicrographs without using, as so many do, old worn-out drawings that show some structures that one can occasionally not identify under the microscope.

He also has a very good chapter on laboratory methods, where he presents a table of various stains and how the different tissues react to them.

The chapter "Morphology of Mesodermal Cells" is clear and well presented.

The photomicrographs vary from very poor to excellent. An illustration of the former is No. 11, representing xeroderma pigmentosum which is little better than a blot of ink.

The book as a whole is not well balanced. The author has over emphasized some diseases, both in the context and photographic illustrations, whereas others are not sufficiently considered. This may be demonstrated by his chapter on leukoplakia, certainly a very important disease, which is not illustrated at all, and Erythroplasia of Queyrat which he puts off with seven or eight lines and no illustrations, stating merely that it represents Bowen's Disease of the glans penis. The truth of this statement is also questionable. By contrast, he devotes three pages and three illustrations to syringocystadenoma papilliferum, which affection I would consider less important than the two preceding.

While most of his descriptions are along conventional lines, he has put forth an interesting classification of epidermal tumors, which, although perhaps not meeting the opinions of all dermatologists, has many things to commend it.

His chapter "Lymphoma and Myelosis" is very well presented and is as clear as is possible for this most confusing group.

On the whole, the book will certainly be helpful to all dermatologists as well as students and perhaps the next edition will remedy some of the faults which in the reviewer's opinion are present.

L. W. K.

Diseases of the Heart. By CHARLES K. FRIEDBERG, M.D. 1081 pp. $11.50. *W. B. Saunders Co., Philadelphia, Pa.*

This book is a very welcome addition to the field of cardiovascular disease. It is printed on good paper. The table of contents is complete enough to find immediately most subjects. At the end of each chapter is an excellent bibliography which is up to date.

The main strength of the book is the clarity and competence with which diseases of the heart have been described in physiological terms. Diseases are described, not so much by the historical statistical clinical method, but by reasoning through the dynamic aspects of the disease processes. Anatomical pathology is not neglected but is presented in conjunction with the functional abnormalities.

Because of its clarity and completeness, this book will serve equally well as a textbook or reference source for student or investigator. It is one of the best books that has been written in the field.

E. V. N.

Streptomycin, Its Nature and Practical Application. Edited by SELMAN A. WAKSMAN. 618 pp. $10.00. *The Williams & Wilkins Co., Baltimore, Maryland.*

Streptomycin has been available in quantity hardly five years, yet here is a large, complete book by some fifty-eight authors on all possible aspects of the chemical nature, commercial production and clinical use of this now well-established antibiotic. The internist, who is faced each day with a decision as to which of four common antibiotics he should use, with or without one of the sulfonamides, must have some source of answer to the question, "what might streptomycin do"? This book goes a long way toward furnishing concrete date on which an answer may be based. For instance, it points out the not too rare use of streptomycin in infections due to gram positive bacteria which have become penicillin resistant, such as staphylococci and streptococcus faecalis. The chapter on pharmacology by Molitor is a particularly clear and lucid one. It is helpful and encouraging in indicating the decreasing toxicity with increasing purification of the drug. The chapter on absorption, distribution and excretion of streptomycin by Rake and Donovick also presents a much needed summary of the moderate amount of knowledge available.

When one then passes from the demonstration of the in vitro effect of streptomycin and the demonstration of efficacy in an experimental infection to the use of the drug in man, one encounters a gap. This is a careful consideration from all viewpoints of how, when and how much drug to give. Dosage schemes in the treatment of tuberculosis are, of course, given and might serve as a model of the kind of thoughtful analysis that is needed. Yet we badly need more careful clinical and experimental evaluation of the need or lack of need for intrathecal administration and use of streptomycin combined with other drugs, to mention but two. This is, of course, mentioned here and there and in the chapter by Spink on brucellosis, the combination with sulfadiazine is given full consideration. The careful analysis of the treatment of non-tuberculous meningitis by Alexander might serve as a model.

The introduction in the past two years of aureomycin and chloromycetin has, of course, somewhat reduced the field of usefulness of streptomycin. The thorough treatment of the use of streptomycin in tuberculosis, where it remains the only practical, useful antibiotic is, therefore, particularly welcome. Experimental evaluation of the drug is given full play, the latest figures on percentage "cures" and types of improvement produced are given in full detail. Yet there is a tendency to pay too much attention to figures based on data of varying values. The discouraging frequency of development of highly resistant strains of tubercle bacilli is clearly presented by Steenken and Wolinsky. The frequency of this occurrence emphasizes the importance of using the drug correctly. Such use can come only with the knowledge contained in such a book as this. The accumulation and careful treatment of this rapidly acquired information is a tribute to the editor of the book and discoverer of the drug, Dr. S. Waksman. The book will be an essential one in any area where an attempt is made to give antibiotics intelligently.

F. B. B.

Acute Appendicitis and Its Complications. By FREDERICK FITZHERBERT BOYCE. 487 pp. $8.75. *Oxford Univ. Press New York,* 1949.

Physicians confronted by this monograph may possibly inquire as to the necessity for a book of nearly 500 pages devoted to a condition which was clearly described by Reginald Fitz more than sixty years ago, and which has since been the subject of thousands of surgical papers and a number of excellent monographs. The author recognizes the grounds for such an opinion but feels that a new presentation is warranted in order to stress the urgency of acute appendicitis. There is no doubt that the "grave and potentially lethal character" of acute appendicitis is well presented here but one wonders if the author might not better have presented his material without such an apologia. If reduction in mortality from this disease is our aim the basic facts of diagnosis and management need to be impressed indelibly on the minds of two groups of people; the practititioners who first see the disease and the public which is afflicted by it. A monographic presentation seems peculiarly unlikely to affect any large proportion of either group.

Having thus caviled at the preface the reviewer finds the remainder of the production eminently satisfactory. There is a fairly detailed historical note which as the author emphasizes, contains a number of facts of great practical value. The next hundred pages cover embryology, anatomy, physiology, bacteriology, pathology and etiology of the disease. The bulk of the volume is properly devoted to diagnosis and treatment, with detailed consideration of the various complications, and special chapters on acute appendicitis in childhood, appendicitis in pregnancy, and appendicitis in relation to trauma. The final chapter on "Factors of Mortality" is an excellent antidote to the smugness concerning appendicitis which is at least partially responsible for the fact that more than five thousand people died of this disease in the U. S. in 1947. We are properly warned against "the lighthearted belief that diagnosis is a simple matter" and against the growing idea in both medical and lay writings that chemotherapy and antibiotics have reduced the mortality from complicated appendicitis to the vanishing point. The thousands of deaths previously noted are convincing testimony to the contrary.

Students will find this an interesting reference work with its value enhanced by a bibliography of some 500 titles and an exhaustive index. The practicing surgeon can find here what is essentially a handbook of diagnosis of acute abdominal conditions. It is unfortunate that the glossy paper detracts from an otherwise excellent format.

C. R. H.

BOOKS RECEIVED FOR REVIEW

An Atlas of the Blood and Bone Marrow. By R. PHILIP CUSTER. 321 pp. $15.00 *W. B. Saunders Co., Philadelphia, Pa.*

A Candle in Her Hand. By DOROTHY GILES. 240 pp. $4.00. *G. P. Putnam's Sons, New York.*

Clinical Electroencephalography. By ROBERT COHN. 639 pp. $14.00. *McGraw-Hill Book Co., New York.*

Handbook of Digestive Diseases, 2nd edition. By JOHN L. KANTOR and ANTHONY M. KASICH. 658 pp. $11.00. *The C. V. Mosby Co., St. Louis, Missouri.*

Normal Values in Clinical Medicine. By F. WILLIAM SUNDERMAN. 845 pp. $14.00. *W. B. Saunders Co., Philadelphia, Pa.*

The Practice of Urology. By HARRY C. ROLNICK, in two volumes. 1245 pp. $24.00. *J. B. Lippincott Co., Philadelphia, Pa.*

Resuscitation and Anesthesia for Wounded Men. By HENRY K. BEECHER. 161 pp. $5.50. *Charles C. Thomas, Publisher, Springfield, Illinois.*

Skin Grafting, new 2nd edition. By JAMES BARRETT BROWN and FRANK MCDOWELL. 339 pp. $7.50. *J. B. Lippincott Co., Philadelphia, Pa.*

Streptomycin and Dihydrostreptomycin in Tuberculosis. Edited by H. MCLEOD RIGGINS and H. CORWIN HINSHAW. *Published by Natl. Tuberculosis Association.*

Surgery of Repair. By JOHN F. PICK. 828 pp. $24.00. *J. B. Lippincott Co., Philadelphia, Pa.*

Volumen Medicinae Paramirum of Theophrastus von Hohenheim Called Paracelsus. By KURT LEIDECKER. 69 pp. $1.75. *The Johns Hopkins Press, Baltimore, Md.*

The Yellow Emperor's Classic of Internal Medicine. By ILZA VEITH. 253 pp. $5.00. *The Williams & Wilkins Co., Baltimore, Md.*

MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

HURD HALL, THE JOHNS HOPKINS HOSPITAL

MONDAY, DECEMBER 12, 1949

Dr. Wilkins: The first paper of the evening will be by Dr. Mark M. Ravitch.

THE OPERATIVE TREATMENT OF PECTUS EXCAVATUM

Pectus excavatum is a congenital and frequently familial defect of the sternum which is usually progressive. It may result in cardiac displacement with resultant embarrassment and diminished exercise tolerance. Cardiac arrhythmias are common. The chest becomes flat and thin with a dorsal kyphos. The cosmetic deformity is a source of embarrassment to the patients.

The operation presented involves the resection of all of the deformed cartilages for the full length of the deformity, the separation of the xyphoid and sternum, and a transverse osteotomy of the sternum at the cranial end of the defect. The sternum is retained in the corrected position with sutures. No traction devices are employed. Paradoxical respiration is not a problem. Twelve children have been treated and two adults. The children vary in age from three months to ten years. There has been one death from postoperative infection. The remaining patients all have satisfactory results. In the twelve children, the operation has been largely prophylactic. Both adults had cardiac displacement producing repeated bouts of arrhythmia in one, and severe cardiac failure with auricular fibrillation in the other. Both have increased exercise tolerance after operation and studies of pulmonary function and cardiac catheterization in the patient with episodes of failure show improvement in pulmonary function and a twofold increase in cardiac output after operation.

Dr. Wilkins: Are there any questions or discussion of Dr. Ravitch's interesting paper? Would you care to add anything, Dr. Blalock?

Dr. Blalock: There are others here who should discuss this paper—Dr. Bing, Dr. McLean, and others. I would simply like to compliment Dr. Ravitch and to listen to a better discussion than I can give.

Dr. Bing: The physiological findings obtained on the patient operated upon by Dr. Mark Ravitch are of interest because they demonstrated a marked reduction of the cardiac output preoperatively followed by a marked rise postoperatively. The systolic pressures in the right ventricle and the pulmonary artery were elevated before the operation and decreased following the surgical correction of the

malformation. The findings in pectus excavatum bring to my mind the similarity in the hemodynamics of this malformation and constrictive pericarditis. In constrictive pericarditis one also finds usually decreased cardiac output and elevated systolic pressures in the right ventricle and in the pulmonary artery. Similar to pectus excavatum after operation the cardiac output increases and the pulmonary arterial pressures fall. This similarity in the findings between the two conditions suggests a common factor responsible for the hemodynamic disturbances. I am thinking here, of a possible obstruction to the outflow from the heart and/or of marked difficulties in emptying of the heart. The latter possibility is more likely in constrictive pericarditis than in pectus excavatum.

I would like to ask Dr. Ravitch whether he has made any observations during the operation which might suggest the presence of such a mechanical obstruction to the outflow from the right ventricle.

Dr. Wilkins: Are there any other questions or discussion? Dr. Andrus?

Dr. Andrus: I don't know how to distinguish between the physiological effects of this kind of deformity and the circulatory disability that results from severe gibbus deformity of the thoracic spine. In the latter, one is accustomed to associated disability with an absolute reduction in the amount of pulmonary volume and pulmonary circulation.

The figures which were displayed tonight for the change in cardiac output referred, I believe, to "cardiac index" (Answer: That is right)—They seem almost fantastically high; the post-operative output was reported as about 7.5 liters. Have there been studies, in these instances, of the pulmonary function as well as the cardiac function?

Dr. Wilkins: Are there any other questions?

Dr. Scott: I would like to ask Dr. Ravitch if he would express his feeling about the possible relationship between pectus excavatum and bronchiectasis. As I recall the operation that ended fatally in his series was a case of a child who did have bronchiectasis. I had an opportunity some years ago to see a child who had a severe pectus excavatum and a total bronchiectasis of the right lung, who had mediastinal and cardiac displacement into the left thorax, and in whom no solution could be found. I would like to know if Dr. Ravitch thinks this deformity predisposes to pulmonary suppuration?

Dr. Nachlas: What happens to the chest wall? Does it stay a weak labile structure or become rigid again?

Dr. Wilkins: Is Dr. Janet Hardy here to make any comments? (Dr. Hardy was not present.) Dr. Ravitch.

Dr. Ravitch: In answer to Dr. Bing's question, we are not in a position, as I think you can probably see, to examine the right ventricle very carefully from direct observation.

In answer to Dr. Andrus, I think this is probably very like the disability in people with Pott's disease and scoliosis. There has been at least one patient who was severely incapacitated by pressure of ribs on the heart in severe scoliosis, who has been operated on by Dr. Lam in Detroit. He simply removed a section of the chest wall with a considerable degree of relief. Dr. McLean did study pulmonary function and perhaps he would care to comment on it. It was his feeling that this man had only a minimal evidence of disability as far as pulmonary function was concerned before operation and that after operation all the changes were in the direction of improvement, none of the magnitude of the changes in the figures which Dr. Bing demonstrated, and which I think he is prepared to back. I may say that the patient is no longer fibrillating. He has been off quinidine and digitalis for two months. His chest is freer and he breathes deeper. He admits to having had no previous disability, except when in cardiac failure before, but now says he has less disability than he had before.

In answer to Dr. Scott's question, it is true that the patient who died had bronchiectasis and for a brief moment we attempted to take shelter under that as the possible source of infection. There is evidence that Lincoln Brown's first case and a patient of Harold Brun's had bronchiectasis. Dr. Lester, in New York has had one or two with bronchiectasis.

This summer, we saw in London, at Great Ormond Street, a child with a nice pectus excavatum, with bronchiectasis of five lobes. He was not operated on for either condition. I don't know what the connection between these two conditions can be. I doubt if there is really a causative connection and one might also add that an appreciable number of the children with cyanotic heart disease and some of the other congenital cardiac conditions have an unusual incidence of chest deformities as Dr. Scott knows better than I, and among those, pectus excavatum.

In answer to Dr. Nachlas' question, I would guess the fracture of the sternum heals in about three weeks and some of the children have developed fairly firm chest walls within that period of time. We brought one child back to a staff conference some months after operation, however, and she still had a fairly springy, leathery chest wall. I rather suspect that it is associated with the amount of perichondrial debris we leave behind. The more debris, the heavier the chest wall after operation.

Dr. Wilkins: The next paper will be a discussion by Dr. Joseph L. Lilienthal, Jr.

STRUCTURE AND FUNCTION OF MUSCLE IN RELATION TO DISEASE

The purpose of the presentation was to review in bare outline some of the more recent and important studies of the intimate structure and the function of the

neuromuscular unit with relation to the role it plays in the pathogenesis of muscular weakness occurring in general medicine. The review included discussion of ultra structure and the more recent studies on muscle proteins associated with contraction and the systems responsible for furnishing energy. There was a brief review of pharmacological reactions of the neuromuscular unit and the sites within the unit where a number of specific diseases exert specific actions. The remainder of the review dealt with distortions in normal states of excitability occurring during disorders of electrolyte metabolism and their various counterparts produced experimentally in the laboratory.

Dr. Wilkins: Dr. Lilienthal has certainly given us plenty of food for thought and his paper should provoke some questions and discussion. Dr. Harvey.

Dr. Harvey: I don't need to reiterate the importance of this whole field and the difficulties attached to working in it. I think all of us have had the experience of knowing how often patients complain of asthenia and I am sure many of us have had an acute infection like influenza and have realized personally the long period of weakness that follows it. I think it is important to point out that the problems involved here as far as eventual goals in relation to clinical medicine are concerned extend far beyond the boundaries of the classical neuromuscular syndromes with which you are familiar.

Dr. Lilienthal has given a very interesting presentation of this difficult field. I am sure you are all impressed with the magnitude of the problems invoved. As one who has worked in this field, I would like to point out that behind this presentation there has gone a great deal of technical skill and very hard work. I have been interested in these problems for the past number of years and Dr. Lilienthal and I have worked together on many of them. Back in 1939 and 1940, I think perhaps I had something to do with stimulating his interest in these problems and I can only say thank goodness he has maintained that interest, because now the field has gotten far too complicated for me and I am glad to retire gracefully.

Dr. Rich: Dr. Wilkins, may I ask Dr. Lilienthal: Since the muscle fibers are bathed in tissue fluid, is anything known about any changes in the cations in the lymph draining a stimulated muscle?

Dr. Lilienthal: I think you have me, Dr. Rich. There are very profound changes, certainly, in potassium concentration of the venous effluent from stimulated muscle. I simply don't know about the lymph.

Dr. Wilkins: Are there any other questions? If not, the meeting is adjourned.